

AMENDMENTS

In the Claims

Please cancel claims 4, 8, 13 and 23 without prejudice or disclaimer.

Please amend claims 1, 5, 10, 14, 17, and 21 as follows:

1. (Amended) A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the steps of:

C1 (a) providing a mammalian cell which comprises a stably integrated AAV cap gene operably linked to a promoter, and a stably integrated AAV rep gene operably linked to a helper virus-inducible heterologous promoter, wherein the AAV cap gene and the AAV rep gene are stably integrated into the mammalian cell's genome, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; and wherein said mammalian cell is prepared by introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into the mammalian cell;

(b) replicating the cell of step (a) to produce a population of cells; and

(c) introducing a helper virus to the population of cells of step (b);

(d) wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

C2 5. (Twice amended) The method according to any of claims 1-3, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

C3 10. (Amended) A mammalian cell for packaging of a recombinant AAV (rAAV) vector, said cell comprising a stably integrated cap gene operably linked to a promoter, and a stably integrated rep gene operably linked to a helper virus-inducible heterologous promoter; wherein the cap gene and the rep gene are stably integrated into the mammalian cell's genome;

C3 wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; wherein said cell exhibits helper-virus-inducible expression of said stably integrated AAV rep gene; and wherein said mammalian cell is prepared by introducing a single plasmid comprising rep and cap arranged as in the AAV genome into the mammalian cell.

C4 14. (Twice amended) The AAV packaging cell of any of claims 10-12, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

17. (Twice amended) A method of packaging a recombinant AAV vector, comprising the steps of:

C5 (a) introducing a helper virus into an AAV packaging cell of claim 15 which comprises a stably integrated rAAV vector comprising a polynucleotide of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and

(b) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

21. (Amended) A method of determining the infectious titer of an rAAV vector preparation, comprising the steps of:

C6 (a) introducing a helper virus and serial dilutions of the rAAV vector preparation to AAV packaging cells of claim 10;

(b) incubating the cells under conditions suitable for replication of AAV; and

(c) determining the amount of replicated rAAV vector relative to an rAAV preparation of known titer.

Please add new claims 24-41.

24. (New) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) preparing an AAV packaging cell according to the method of claim 1, wherein the AAV packaging cell exhibits helper virus-inducible expression of the stably integrated AAV rep gene;

(b) introducing a recombinant AAV (rAAV) vector into the AAV packaging cell, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter; and

(c) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

25. (New) A method of producing a mammalian cell for packaging of a recombinant AAV (rAAV) vector, said method comprising the step of:

(a) introducing a single plasmid comprising AAV rep and AAV cap arranged as in the AAV genome into a mammalian cell, wherein the AAV cap gene is operably linked to a promoter and the AAV rep gene is operably linked to a helper virus-inducible heterologous promoter, wherein p5 promoter function has been replaced by the helper virus-inducible heterologous promoter; wherein the plasmid becomes stably integrated into the mammalian cell's genome; and wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

26. (New) The method according to claim 25, wherein said helper virus is an adenovirus.

27. (New) The method according to claim 25, wherein said packaging cell grows at least one half as rapidly as parental-type cells that do not contain an AAV rep gene, and wherein said packaging cell when used to package rAAV vectors produces at least 100 rAAV particles/cell.

28. (New) The method according to any of claims 25-27, wherein said heterologous promoter is a mouse metallothionein I (mMT-I) promoter.

29. (New) A cell produced by the method of claim 27, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

30. (New) A cell produced by the method of claim 25, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

31. A cell produced by the method of claim 28, and progeny thereof, wherein said cell exhibits helper virus-inducible expression of said stably integrated AAV rep gene.

C9 32. (New) A cell produced by the method of claim 25, and progeny thereof, wherein said cell further comprises a stably integrated recombinant AAV (rAAV) vector, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter.

33. (New) A method of packaging a recombinant AAV vector, comprising the steps of:

(a) introducing a recombinant AAV (rAAV) vector into the AAV packaging cell of claim 29, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;

(b) introducing helper virus; and

(c) incubating the cell under conditions suitable for replication and packaging of AAV such that rAAV vector is packaged.

34. (New) A method of packaging a recombinant AAV vector, comprising the steps of:
(a) introducing a helper virus into an AAV packaging cell of claim 32; and
(b) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

35. (New) The method of claim 1, wherein the AAV cap gene is operably linked to AAV p40 promoter.

36. (New). The method of claim 24, wherein the AAV cap gene is operably linked to AAV p40 promoter.

CM 37. (New) The cell of claim 10, wherein the AAV cap gene is operably linked to AAV p40 promoter.

38. (New) A method of packaging a recombinant AAV vector, comprising the steps of:
(a) preparing an AAV packaging cell according to the method of claim 25, wherein the AAV packaging cell exhibits helper virus-inducible expression of the stably integrated AAV rep gene;

(b) introducing a recombinant AAV (rAAV) vector into the AAV packaging cell, said vector comprising a polynucleotide sequence of interest located between two AAV inverted terminal repeat (ITR) regions, wherein said polynucleotide is operably linked to a promoter;

(c) introducing a helper virus; and

(d) incubating the cell under conditions suitable for replication and packaging of AAV such that said rAAV vector is packaged.

39. (New) The method of claim 33, wherein said helper virus is an adenovirus.

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40. (New) The method of claim 34, wherein said helper virus is an adenovirus.

41. (New) The method of claim 38, wherein said helper virus is an adenovirus.
